

WE CLAIM:

1. A modified catalase polypeptide having a carboxy-terminal peroxisome targeting signal (PTS) that has been modified from a native sequence of Lys-Ala-Asn-Leu (SEQ ID NO: 1) by replacement with a PTS comprising the sequence Xaa<sub>3</sub> - Xaa<sub>2</sub> - Xaa<sub>1</sub>, wherein, independently,

5 Xaa<sub>3</sub> is Ser, Ala or Cys;  
Xaa<sub>2</sub> is Lys, Arg or His; and  
Xaa<sub>1</sub> is Leu or Met.

2. The modified catalase polypeptide of claim 1, further comprising, to the amino-terminal side of Xaa<sub>3</sub>, *n* additional amino acid residues wherein *n* is an integer between 1 and about 17, the additional residues being numbered sequentially from Xaa<sub>4</sub> for the first additional residue to Xaa<sub>20</sub> for the seventeenth additional residue.

3. The modified catalase polypeptide of claim 2, wherein *n* is between about 5 and about 17

4. The modified catalase polypeptide of claim 3, wherein *n* is between about 7 and about 13.

5. The modified catalase polypeptide of claim 3, wherein *n* is between about 9 and about 11.

6. The modified catalase polypeptide of claim 3, wherein *n* is 9.

7. The modified catalase polypeptide of claim 1, wherein *n* is at least 1, 2 or 3, and residues at any one of Xaa<sub>6</sub> to Xaa<sub>4</sub> are hydrophobic amino acids.

8. The modified catalase polypeptide of claim 7, wherein residues at any one of Xaa<sub>6</sub> to Xaa<sub>4</sub> are, independently, Leu, Val, Ile, Ala or Gly.

9. The modified catalase polypeptide of claim 1, wherein *n* is at least 1, and residue Xaa<sub>4</sub> is a negatively charged amino acid.

10. The modified catalase polypeptide of claim 9, wherein residue Xaa<sub>4</sub> is Lys, Arg or His.

11. The modified catalase polypeptide of claim 10, wherein residue Xaa<sub>4</sub> is Lys.

12. The modified catalase polypeptide of any of claims 1-11, wherein Xaa<sub>3</sub> is Ser, Xaa<sub>2</sub> is Lys, and Xaa<sub>1</sub> is Leu.

13. A modified catalase polypeptide, which comprises, at or near its amino-terminus, an amino acid sequence comprising the PTS2-type sequence (Arg/Lys)-(Leu/Ile/Val)-(X<sub>5</sub>)-(His/Gln)-(Ala/Leu/Phe).

14. The modified catalase polypeptide of claim 13, wherein the PTS2-type sequence is Arg-Leu-Gln-Val-Val-Leu-Gly-His-Leu (SEQ ID NO: 11).
15. The modified catalase of claim 1, which further comprises, at or near its amino-terminus, an amino acid sequence comprising the PTS2-type sequence (Arg/Lys)-(Leu/Ile/Val)-(X<sub>5</sub>)-(His/Gln)-(Ala/Leu/Phe).
16. The modified catalase of claim 15, wherein the PTS2-type sequence is Arg-Leu-Gln-Val-Val-Leu-Gly-His-Leu (SEQ ID NO: 11).
17. A nucleic acid molecule encoding the modified catalase polypeptide of claim 1, 2, 13 or 15, wherein the coding sequence is operably linked to an expression control sequence.
18. A host cell comprising the polynucleotide of claim 17.
19. A method for preparing the modified catalase of claim 1, 2, 13 or 15, comprising
- (a) incubating a host cell that comprises a nucleic acid encoding said modified catalase polypeptide under conditions effective for expression of said polypeptide, and
  - (b) harvesting the modified catalase from the host cell.
20. A pharmaceutical composition comprising:
- (a) the modified catalase polypeptide of any of claims 1-11; and
  - (b) a pharmaceutically acceptable excipient or carrier.
21. A pharmaceutical composition comprising:
- (a) the modified catalase polypeptide of claim 12; and
  - (b) a pharmaceutically acceptable excipient or carrier.
22. A pharmaceutical composition comprising
- (a) the modified catalase polypeptide of any of claim 13 or 15; and
  - (b) a pharmaceutically acceptable excipient or carrier.
23. A deliverable, peroxisomally-targeted polypeptide comprising:
- (a) the modified catalase polypeptide of any of claims 1-11, and
  - (b) a delivery or translocation molecule or moiety bound thereto or associated therewith.
24. A deliverable, peroxisomally-targeted polypeptide comprising:
- (a) the modified catalase polypeptide of claim 12, and
  - (b) a delivery or translocation molecule or moiety bound thereto or associated therewith.

25. A deliverable, peroxisomally-targeted polypeptide comprising:

- (a) the modified catalase polypeptide of any of claims 13 or 15, and
- (b) a delivery or translocation molecule or moiety bound thereto or associated therewith.

5 26. The deliverable, peroxisomally targeted polypeptide of claim 23, wherein the delivery molecule is a peptide or polypeptide.

27. The deliverable, peroxisomally targeted polypeptide of claim 24, wherein the delivery molecule is a peptide or polypeptide.

10 28. The deliverable, peroxisomally targeted polypeptide of claim 25, wherein the delivery molecule is a peptide or polypeptide.

29. The deliverable polypeptide of claim 26 wherein the peptide or polypeptide is selected from the group consisting of

- (a) HIV-TAT protein or a translocationally active derivative thereof,
- (b) penetratin having the sequence RQIKIWFQNRRMKWKK (SEQ ID NO: 4),
- 15 (c) a penetratin variant W48F having the sequence RQIKIFFQNRRMKWKK (SEQ ID NO: 5)
- (d) a penetratin variant W56F having the sequence RQIKIWFQNRRMKFVK, SEQ ID NO: 6)
- (e) a penetratin variant having the sequence RQIKIWFQNRRMKFVK, SEQ ID NO:
- 20 7)
- (f) herpes simplex virus protein VP22 or a translocationally-active homologue thereof from a different herpes virus; and
- (g) Pep-1, having the sequence KETWWETWWTEWSQPKKKRKV (SEQ ID NO: 9).

25 30. The deliverable polypeptide of claim 27 wherein the peptide or polypeptide is selected from the group consisting of

- (a) HIV-TAT protein or a translocationally active derivative thereof,
- (b) penetratin having the sequence RQIKIWFQNRRMKWKK (SEQ ID NO: 4),
- (c) a penetratin variant W48F having the sequence RQIKIFFQNRRMKWKK (SEQ
- 30 ID NO: 5)
- (d) a penetratin variant W56F having the sequence RQIKIWFQNRRMKFVK, SEQ ID NO: 6)

- (e) a penetratin variant having the sequence RQIKIWFQNRRMKFKK, SEQ ID NO: 7)
- (f) herpes simplex virus protein VP22 or a translocationally-active homologue thereof from a different herpes virus; and
- (g) Pep-1, having the sequence KETWWETWWTEWSQPKKKRKV (SEQ ID NO: 9).

31. The deliverable polypeptide of claim 28 wherein the peptide or polypeptide is selected from the group consisting of

- (a) HIV-TAT protein or a translocationally active derivative thereof,
- (b) penetratin having the sequence RQIKIWFQNRRMKWKK (SEQ ID NO: 4),
- (c) a penetratin variant W48F having the sequence RQIKIFFQNRRMKWKK (SEQ ID NO: 5)
- (d) a penetratin variant W56F having the sequence RQIKIWFQNRRMKFKK, SEQ ID NO: 6)
- (e) a penetratin variant having the sequence RQIKIWFQNRRMKFKK, SEQ ID NO: 7)
- (f) herpes simplex virus protein VP22 or a translocationally-active homologue thereof from a different herpes virus; and
- (g) Pep-1, having the sequence KETWWETWWTEWSQPKKKRKV (SEQ ID NO: 9).

32. The deliverable polypeptide of claim 29, wherein the delivery molecule is Pep-1.

33. The deliverable polypeptide of claim 23, wherein the delivery moiety associated with the modified catalase is a liposome.

34. The deliverable polypeptide of claim 33 wherein the liposome comprises effective concentrations of external membrane phosphatidylserine for uptake by phagocytic cells or other phosphatidylserine-recognizing cells.

35. A method for reducing the concentration of hydrogen peroxide in a cell, comprising contacting said cell with a modified catalase polypeptide of any of claims 1-11 and 13-16, under conditions wherein said polypeptide is targeted to peroxisomes in an amount sufficient to reduce said concentration.

36. The method of claim 35, wherein the modified catalase polypeptide further comprises a delivery or translocation molecule or moiety bound thereto or associated therewith.

37. The method of claim 35, wherein the contacting is *in vitro*.
38. The method of claim 35, wherein the contacting is *in vivo*.
39. The method of claim 37, wherein the cell is a stem cell.
40. The method of claim 37, wherein the cell is part of an artificial organ.
- 5 41. The method of claim 36, wherein the contacting is *in vitro*.
42. The method of claim 36, wherein the contacting is *in vivo*.
43. The method of claim 41, wherein the cell is a stem cell.
44. The method of claim 41, wherein the cell is part of an artificial organ.
45. A method for treating a mammalian subject suffering from a disease or condition
- 10 associated with or caused by an inadequate level of peroxisomally active catalase, comprising administering to the subject an effective amount of the modified catalase polypeptide of any of claims 1-11 and 13-16.
46. A method for treating a subject suffering from a disease or condition associated with or caused by an inadequate level of peroxisomally active catalase, comprising administering to the
- 15 subject an effective amount of the pharmaceutical composition of claim 20.
47. A method for treating a subject suffering from a disease or condition associated with or caused by an inadequate level of peroxisomally active catalase, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 21.
48. A method for treating a subject suffering from a disease or condition associated with or
- 20 caused by an inadequate level of peroxisomally active catalase, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 22.
49. The method of claim 45, wherein the subject is a human.
50. The method of claim 45, wherein the disease or condition is age-related.
51. A method treating for preventing the development of age-related skin wrinkling or other
- 25 disfigurement, comprising carrying out the method of claim 45.
52. The method of claim 45 wherein said administering is topical.
53. The method of claim 45, wherein the disease or condition is hyperlipidemia, a skin disease, a neurodegenerative disease, an existing ischemic condition or a risk of reperfusion injury subsequent to treatment of the ischemic condition.
- 30 54. The method of claim 53 wherein said administering is topical.
55. The method of claim 45, wherein the subject is an agricultural animal or a cloned animal.